1991

# Uterine position effects in CF-1 and CK female mice

Athena E. Cologer-Clifford
*Lehigh University*

# Uterine Position Effects in CF-1 and CK Female Mice

by

Athena E. Cologer-Clifford

A Thesis

Presented to the Graduate Committee

of Lehigh University

in Candidacy for the Degree of

Masters of Science

in Psychology

Lehigh University

May 1991

This thesis is accepted and approved in partial fulfillment of the requirements for the Masters of Science.

5/13/91
Date

Professor N.G. Simon
Thesis Advisor

Professor M.L. Richter
Co-Advisor

Professor J.G Nyby
Co-Advisor
Chairman of Department

Aknowledgements:

I sincerely thank Dr. Neal G. Simon for his help and support in enabling me to grow and directing my education toward greater personal and professional enrichment. I also extend my appreciation to Dr. John Nyby and Dr. Martin Richter for their comments and suggestions.

Table of Contents


Abstract . . . . . 1

I. General Introduction . . . . . 3

II. General Methods . . . . . 16-19

1. Animals . . . . . 16

2. Mating and Delivery . . . . . 17

3. Surgery and Aggression Tests . . . . . 18

III. Experiment I . . . . . 20-24

1. Methods . . . . . 20

2. Results . . . . . 20

3. Discussion . . . . . 23

IV. Experiment II . . . . . 24-36

1. Methods . . . . . 24

2. Results . . . . . 28

3. Discussion . . . . . 32

V. General Discussion . . . . . 37

VI. References . . . . . 40

VII. Appendices - Plates . . . . . 44

Vita. . . . . 48

List of Figure and Tables

Figure 1: Classification of female mouse fetuses based on position relative to males during uterine development . . 19

Table 1: Experiment 1 - Aggressive behavior exhibited by CK and CF-1 female mice in response to testosterone implants . . . . . 21

Table 2: Experiment 1 - Aggressive behavior exhibited by 0M, 1M, and 2M CF-1 female mice in response to testosterone implants . . . . . 21

Table 3: Experiment 1 - Aggressive behavior exhibited by 0M, 1M, and 2M CK female mice in response to testosterone implants . . . . . 22

Table 4: Experiment 1 - Number of days of testosterone treatment required to activate aggression in 1M CF-1 and CK female mice - Comparison between females contiguous to either a rostral or caudal male during uterine development (1R vs. 1C) . . . . . 23

Table 5: Experiment 2 - Ano-genital distance to body weight ratio in 0MN, 1M, and 2M CF-1 female mice . . . . 28

Table 6: Experiment 2 - Age of vaginal opening and estrous cycle lengths exhibited by 0M, 1M, and 2M CF-1 female mice . . . . . 29

Table 7: Experiment 2 - Aggressive behavior exhibited by 0M, 1M, and 2M CF-1 female mice following testosterone treatment . . . . . 30

Table 8: Experiment 2 - Comparison of 1M CF-1 female mice - Females contiguous to either a rostral or caudal male during uterine development (1R vs. 1C) . . . . . 31

Table 9: Experiment 2 - Androgen binding in the hypothalamic-preoptic-septal region of 0M, 1M, and 2M CF-1 female mice following testosterone treatment . . . . . 32

# List of Plates

Plate 1: Estrus Phase . . . . . 44

Plate 2: Metestrus Phase . . . . . 45

Plate 3: Diestrus Phase . . . . . 46

Plate 4: Proestrus Phase . . . . . 47

## ABSTRACT

The relative position of a female rodent with respect to male littermates during uterine development presumably accounts for a range of individual differences observed in females. Evidence in support of this theory, referred to as the uterine position phenomenon, has been presented in a number of rodents, particularly rats and mice. However, specific uterine position effects are limited to single studies or to a specific rodent strain or species. It was the purpose of this study to replicate the effects of uterine position on physiological measures in CF-1 female mice and extend behavioral evidence for differences in sensitivity to testosterone from Rockland-Swiss (RS) female mice to CF-1 and CK females.

Previous findings suggested that females contiguous to two males (2M) during uterine development displayed delayed vaginal opening with increased estrus cycle lengths and larger ano-genital distance to body weight ratios at birth when compared with females contiguous to two females (0M). The current study was unable to replicate these results, finding no differences between 0M and 2M CF-1 females on any of these measures. Further, the behavioral data in CF-1 and CK female mice were not consistent with the uterine position model. 2M RS females were reported to have a higher sensitivity to the aggression-promoting property of testosterone (T) than 0M females, requiring a shorter duration of T treatment to activate aggressive behavior against a male conspecific. In contrast, 0M and 2M CF-1 and CK females required similar T treatment durations to activate aggression. Consistent with the behavioral data in this study, 0M and 2M CF-1 adult females had similar levels of androgen binding in response to T treatment, suggesting that there were no induced differences as a result of uterine

position in the underlying biochemical mechanisms thought to mediate the expression of aggressive behavior.

The findings from the current study question the general utility of the uterine position phenomenon to explain individual variation among female rodents. At least two possible explanations may account for the discrepency in the findings. The first concerns the fact that while the perinatal period is critical for the development and differentiation of physiological and behavioral parameters, this model focuses only on the prenatal environment. Differences in parameters whose development occur largely postnatally would therefore not be affected by uterine position. The second explaination suggests that there are genetic variations among rodent strains and species which limit the extent to which uterine position influences development and subsequent morphological and behavioral expression.

# INTRODUCTION

Individual variation is a natural and necessary phenomenon which helps insure the survival and evolution of a species. Genetic mechanisms, such as mutation and recombination, as well as environmental and hormonal factors contribute to the physiological and behavioral diversity expressed by members of a species. Female rodents, for instance, display differences in a range of physiological measures. Some females spontaneously display male-typical behaviors, such as mounting (Beach, 1971; Clemens, 1974) or heightened sensitivity to the activational effects of androgens on these behaviors in adulthood (Gandelman, vom Saal, and Reinisch, 1977; Clemens, Gladue, and Coniglio, 1978; Quadagno, McQuitty, McKee, Lowlliker, Wolfe, and Johnson, 1987). Further, differences in the onset of puberty and subsequent estrous cycle lengths also have been observed (vom Saal, Pryor, and Bronson, 1981; Rines and vom Saal, 1984; Clark and Galef, 1988). While genetic factors may contribute to the expression of these differences, the development and expression of many of these behaviors are hormonally determined during sexual differentiation.

Sexual differentiation is primarily regulated by the absence or presence of testicular hormones. Thus in males, testosterone secreted from the testes induces undifferentiated peripheral target tissues to express the male phenotype, and causes neural tissues to become more sensitive to testosterone, i.e. brain regions mediating the expression of masculine behaviors are easily activated by exposure to testosterone in adulthood. In contrast, the lack of perinatal testosterone exposure produces a phenotypic female, with brain regions regulating the expression of masculine behaviors less sensitive to the activational effects of testosterone. It is important to note that this model of sexual differentiation allows for the development of neural

networks regulating the expression of both masculine and feminine behaviors in each sex. However, the sensitivity of these neural substrates to the activating steroid in adulthood differs based on perinatal exposure to the steroid. Thus, a natural mechanism which may cause some females of a species to be exposed to testosterone prenatally may contribute to slight masculinization of peripheral and neural target tissues and help explain individual differences in morphology and behavior.

In 1971, Clemens and Coniglio reported a positive relationship between the proportion of adult female rats that mounted after ovariectomy and testosterone treatment and the number of male littermates. In 1974, Clemens reported that female rats developing contiguous to two males (2M) in the uterus had longer ano-genital distances than females developing contiguous to one male (1M) or two females (0M). Clemens was also able to eliminate this difference by exposing the pregnant mothers to the antiandrogen Sch 13521 (4' nitro-3-trifluoromethylisobutyranilide). Further, although the findings were not significant, Clemens found that 2M adult females displayed a higher frequency of mounting behavior when ovariectomized and given testosterone than 1M females or females from all female litters. The findings of individual differences among female rats in morphology and sensitivity to testosterone, coupled with the antagonism of these effects by antiandrogen treatment, suggested that female fetuses were being exposed to testosterone prenatally, possibly secreted by contiguous male littermates. Since this early work, support for a uterine position phenomenon as a mechanism to explain individual differences has been presented in several rodent species and across a range of physiological and behavioral measures.

Clemens' contiguity hypothesis (1974) thus presented a potential model for explaining slight variations in the morphology and behavior of female

rats based on the number of contiguous male littermates. The primary emphasis of this model was on direct contiguity to males. Thus males on either side of the female during uterine development were sources of prenatal testosterone exposure. Subsequent research, however, suggested an alternate model for the differential masculinization of female rats (Meisel and Ward, 1981). Meisel and Ward (1981) suggested that contiguity per se was not of primary importance, but the testosterone secreted by a male on the caudal, or cervical, end of the uterine horn (1C) played the decisive role. Testosterone secreted by males located on the rostral, or ovarian, end of the uterine horn (1R) did not influence the development of the female (1R females). It was reported for ovariectomized androgen treated females paired with a sexually receptive female, a higher frequency of mounting behavior for 1C females when compared with females having no males on the caudal side (Meisel and Ward, 1981). However, under such a classification scheme it remained possible for some of the 1C females to be classified as 2M females, thus making a clear comparison of the two theories difficult. Direct support for the caudal hypothesis is further obscured by findings that 2M females displayed a higher frequency of mounting behavior when compared with 0M females, that support the contiguity model. However, stronger evidence in support of Meisel and Ward's caudal hypothesis in rats stemmed from comparisons between 1M females initially classified according to the contiguity model and then divided into 1R or 1C females. Under this classification paradigm, 1C females displayed a significantly higher frequency of mounting than 1R females (Meisel and Ward, 1981). However, unlike the contiguity hypothesis, where a positive relationship between the number of contiguous males and the degree of masculinization in female rats exists, Meisel and Ward (1981) found no differences in the degree of masculinization

in females with one or more caudal males. Thus, while the specific mechanisms differ, both models recognize a degree of masculinization induced by prenatal exposure to T secreted by male littermates

The primary difference between the contiguity (Clemens, 1974) and caudal (Meisel and Ward, 1981) hypotheses lies in the region of the uterus where testosterone secreted by the male littermates can diffuse and enter the female's blood supply. The contiguity hypothesis suggests that diffusion of testosterone occurs across the amniotic sacs, while the caudal hypothesis suggests that testosterone diffuses across the venous and arterial blood vessels. According to the latter model, arterial and venous blood both flow from the uterus to the ovaries and the two vessels are close enough to allow hormones to pass between them. Thus testosterone entering the venous blood from males located caudal to the female diffuses into the arterial vessel suplying blood to the female. Although the two theories are not mutually exclusive, tracing the flow of blood in the rodent uterus would help resolve this issue and provide a basis for establishing the mechanism underlying the presumed uterine position model.

While the primary mechansim for uterine position effects lies in differential exposure to testosterone, studies examining the concentration of testosterone in the blood and amniotic fluid of fetal female rodents from different uterine positions have been inconclusive. vom Saal and Bronson (1980) found that 2M CF-1 female mice had higher testosterone levels in both the blood and amniotic fluid than 0M females on day 17 of gestation. However, following a similar methodology, no differences in testosterone levels were found in the serum of female rats on day 20 of gestation (Slob, Ooms, and Vreeburg, 1978), or in the serum and amniotic fluid of female hamsters on day 14 of gestation (Vomachka and Lisk, 1986). Closer

examination of the three studies reveals a much higher concentration of testosterone in the blood serum of mice, than in either the blood serum of hamsters or rats, which may have facilitated the detection of slight concentration differences in female mice from the different uterine positions. Female mice on average had approximately 980 ± 50 pg/ml testosterone in serum samples (vom Saal and Bronson, 1980), while female rats had approximately 120 ± 45 pg/ml (Slob, Ooms and Vreeburg, 1978), and female hamsters had only approximately 10 ± 5 pg/ml (Vomachka and Lisk, 1986). Male mice had average plasma testosterone concentrations of 3000 ± 140 pg/ml, male rats 680 ± 120 pg/ml, and male hamsters approximately 120 ± 10 pg/ml. These serum T levels reported for mice (vom Saal and Bronson, 1980) are similar to more recent measures of serum T levels for male and female mice, 1973 pg/ml and 865 pg/ml respectively (Motelica-Heino, Castanier, Corbier, Edwards, and Roffi, 1988).

When comparing the large difference in plasma testosterone concentrations between male and female mice, it is questionable whether a difference of 200 pg/ml found between 0M and 2M CF-1 females is sufficient to account for the more pronounced morphological and behavioral differences reported between these two classifications. Further, while not significant, there was a tendency for female rats from a high female:low male litter ratio to have higher plasma testosterone concentrations than female rats from low female:high male litter ratio, a finding which is not consistent with the uterine position model (Slob, Ooms, and Vreeburg, 1978). In addition, the low plasma testosterone concentration in male and female hamsters suggests that the testosterone surge from male testes contributing to sexual differentiation may occur postnatally. In fact, there is support for masculinization of male hamsters occurring in the first few days of postnatal

life (Paup, Coniglio, and Clemens, 1974; Coniglio, Paup, and Clemens, 1973), and thus the utility of uterine position as a mechanism for contributing to individual variation in female hamsters is limited. The data from the testosterone assays have not provided clear evidence that uterine position causes differential exposure among female fetuses to testosterone and raises questions regarding the general applicability of this model across rodent species.

Despite inconsistencies in the fetal testosterone assay data, alternative measures have been examined as an index of androgen exposure. For example, ano-genital distance, the distance of the androgen sensitive tissue between the anal opening and the genital region, has served as an early marker of prenatal masculinization. Prenatal exposure to testosterone induces growth in this tissue, thus increasing the distance between the anus and genitalia. Gandelman, vom Saal and Reinisch (1977) reported a longer ano-genital distance in 2M Rockland-Swiss female mice when compared to 0M females. However, this difference did not take into account differences in body weight between the two groups of females which is necessary since heavier animals also have longer ano-genital distances. Further, findings that 2M Rockland-Swiss females were heavier than 0M females (Kinsley, Meile, Wagner, Ghiraldi, Broida, and Svare, 1986) make it difficult to interpret the effects of uterine position on ano-genital distance in Rockland-Swiss mice. On the other hand, taking body weight into account, 2M CF-1 female mice had a longer ano-genital distance than 0M females (vom Saal and Bronson, 1978) and this difference was still present when the females' ano-genital distance:body weight ratios were remeasured at 60 days of age. Several studies have also examined the effects of uterine position on differences in ano-genital distance to body weight ratios in Sprague-Dawley

female rats. Following the contiguity hypothesis, 2M females had a larger ano-genital distance to body weight ratio than 0M females (Clemens, 1974; Meisel and Ward, 1981; and Richmond and Sachs, 1984). Further, in support of the caudal hypothesis, 1C female rats had a larger ano-genital distance to body weight ratio than 1R females (Meisel and Ward, 1981, and Richmond and Sachs, 1984). Based on the available evidence, ano-genital distance to body weight ratio has been a consistent indicator of prenatal testosterone exposure.

While studies examining ano-genital distance support the uterine position phenomenon as a mechanism for generating morphological differences among female mice and rats, it is also important to examine the effects of this model on other physiological responses to determine its general utility. Such measures have included preference behavior, onset and length of estrus cycles, and capacity to produce live litters. vom Saal and Bronson (1978) have reported a preference for 0M CF-1 females over 2M females by male mice. This measure was defined by the goal box, containing either a 2M or 0M female, entered by the male after exploring both boxes. The authors suggested that this preference translated into an increased likelihood for 0M females to mate over 2M females. However, studies investigating females' readiness to mate, by examing onset of puberty and estrus cycle lengths, have found variable results depending on the age and housing conditions of the animal. For instance, 0M CF-1 females entered puberty, as assessed by the time of the first estrus cycle, sooner than 2M females when individually housed in the presence of a male, but not when group housed in the presence of a male (vom Saal, 1981). In addition, while this first estrus cycle was longer in 0M females when compared with 2M females, the cycles became shorter

than 2M females in adulthood when group housed in the presence of a male or individually housed in the absence of a male.

The subtlety of effects on estrous cycles and preference behavior may be due to the influence of pheromonal cues in general rather than intrinsic differences attributable to uterine position. It is well established that female mice are sensitive to pheromonal cues from both males and females and that this may cause estrus cycles to vary in length and regularity (Vandenbergh, 1967; Vandenbergh, 1974; Stiff, Bronson, and Stetson, 1974; Drickamer, 1977). For instance, while it was found that 0M females delivered the last litter containing live young at an older age than 2M females, and also delivered more live litters over their reproductive lifespan when compared with 2M females (vom Saal and Moyer, 1985), the findings were obtained by continually individually housing a stud male with a female once the previous litter was weaned. This eliminated the effects of competition and pheromonal cues which play a major role in reproduction under natural conditions. In fact, when CF-1 females were group housed with a male for twenty days after the onset of puberty, there were no differences in the proportion of 0M and 2M females which became impregnated (vom Saal and Bronson, 1978).

In addition, Clark and Galef (1988) examined the effects of uterine position on the rate of sexual maturation in mongolian gerbils by measuring the age of vaginal opening. They reported that first vaginal opening occurred at an earlier age in 0M females than in 2M females. However, this data is confounded by the fact that early maturing gerbils (quicker onset of vaginal opening and delivery of the first litter) are born in predominantly large female litters, while late maturing gerbils are born in smaller, predominantly male litters (Clark, Spencer, and Galef, 1986; Clark and Galef, 1988). Thus, 0M

females with an earlier onset of vaginal opening came from large female litters, while 2M females with a later onset of vaginal opening came from small male litters (Clark and Galef, 1988). Further, a comparison between uterine positions within litters found that in 10 of the 19 litters there were no differences in onset of vaginal opening between 0M and 2M females. However, where differences were observed in 8 of the litters, 0M females displayed earlier onset of vaginal opening (Clark and Galef, 1988).

In sum, the effects of uterine position on reproductive capacity in female rodents are not clear. It has been argued that contiguity to two males during uterine development results in delayed onset of puberty and lengthened estrus cycles in female rodents (vom Saal, 1981; vom Saal, 1983). While under particular housing conditions such differences between 0M and 2M females have been demonstrated in CF-1 mice, housing conditions do alter the timing of these patterns, suggesting that pheromonal cues play a major role in these reproductive cycles. In addition, the onset of puberty in female gerbils is best predicted by litter composition as a whole rather than direct contiguity to male littermates. These findings suggest that delays in puberty and lengthened estrus cycles are not soley regulated by contiguity to males during prenatal development, and raise questions regarding the degree to which uterine position contributes to individual differences in reproductive capacities among female rodents.

Another characteristic reportedly affected by uterine position is the display of male-typical behaviors in response to testosterone treatment in adulthood. These studies have involved ovariectomy and treatment with testosterone, either through implanting silastic capsules or daily injections, followed by behavioral testing for aggression and/or mounting. Early evidence for a relationship between the effects of male littermates on differences in

sensitivity to testosterone among females came from a study of mounting behavior in rats by Clemens and Coniglio (1971). These researchers found that a greater proportion of female rats from higher male to female litter ratios mounted when ovariectomized and treated with testosterone in adulthood than females from lower male to female ratios. However, Clemens and Coniglio (1971) found no differences in the frequency of mounting behavior due to litter composition; a finding also supported by Slob and van der Schoot (1982) in Wistar rats. Subsequent research by Clemens, Gladue, and Coniglio (1978) found that classifying female rats based on their contiguity to males in the uterus resulted in differences, with 2M females displaying a higher frequency of mounting behavior than 1M or 0M females when ovariectomized and treated with testosterone. Meisel and Ward (1981) also found that 2M Sprague-Dawley female rats displayed a higher frequency of mounting behavior than 0M females when given testosterone in adulthood. These researchers further examined the 1M females, dividing them into females with a caudal male (1C) and females with a rostral male (1R). Meisel and Ward found that 1C females had a higher frequency of mounting behavior than 1R females, and there was no difference between 1C and 2M females. In addition, Gandelman (1986) examined the frequency of mounting behavior in female guinea pigs, using both contiguity and caudal classification schemes. Gandelman (1986) found that while 2M and 1C females did not differ, both had a higher frequency of mounting behavior than 0M females. Further, 1C females displayed a higher frequency of mounting behavior than 1R females. These findings from rats and guinea pigs present a fairly consistent pattern of differences between females in their sensitivity to testosterone's activation of mounting behavior based on their uterine position. The evidence suggests that testosterone

secreted by caudal males during uterine development can partially masculinize the neural substrate regulating mounting behavior, and that contiguity alone is not sufficient.

In contrast, research examining the effects of uterine position on the activation of mounting behavior by testosterone in female mice has produced inconsistent results. vom Saal and Bronson (1978) found no difference in the proportion of 0M and 2M CF-1 female mice which mounted sexually receptive females after testosterone treatment and weekly testing for 4 weeks. However, a later study by Rines and vom Saal (1984) following a similar methodology found that a higher proportion of 2M CF-1 females mounted a sexually receptive female when compared with 0M females. While these investigations examined behavior shortly after testosterone treatment for a period of 4 weeks, additional studies examining mounting behavior in mice have employed two behavioral tests after extended treatment periods. For instance, Quadagno and coworkers (1987) using a DBA/2 x C57Bl/6 hybrid, found that a higher proportion of 2M females mounted when tested 34 and 36 days after testosterone implants. On the other hand, Gandelman and Kozak (1988) found no difference in the proportion or frequency of 0M and 2M Rockland-Swiss females that mounted a sexually receptive female 22 and 42 days after testosterone implants. Interpretation of the results are difficult since there a methodological and strain variations. While these observations suggest that uterine position effects may be genetically constrained, the inconsistent findings with CF-1 females and the negative results in Rockland-Swiss mice suggest an alternative hypothesis. Gandelman and Kozak (1988) found that treating CF-1 female mice on the day of birth with a single injection of 500 ug testosterone propionate caused them to exhibit mounting behavior sooner and more frequently than 0M or 2M females. This suggests

that differentiation of male sexual behavior in mice may occur largely postnatally thus limiting the effects of prenatal testosterone exposure due to uterine position on the expression of this behavior.

Another model used to examine responsiveness to testosterone in female mice has been aggressive behavior. However, because the stimulus animals have varied, employing either sexually receptive females or testosterone treated females, the resulting aggressive response can not be viewed as regulated by similar neural mechanisms. Thus different forms of aggressive behavior have been elicited depending on the stimulus animal. Further, in accord with research on mounting behavior, different treatment durations have also contributed to the difficulty in developing a consistent model for the effects of uterine position on the expression of aggressive behavior. Quadagno et al. (1987) tested aggression in CF-1 females by pairing testosterone treated females of the same uterine position and measuring the proportion of females from each uterine position fighting. Two tests conducted 48 hours apart after 25 days of testosterone implants found that a higher proportion of 2M females displayed aggressive behavior when compared with 0M females. Again pairing testosterone treated females, but testing once a week for 4 weeks after implanting testosterone capsules, Rines and vom Saal (1984) also found that a higher proportion of 2M CF-1 females than 0M females fought, although no differences were found in the frequency or latency to attack. Using a different behavioral paradigm by testing for aggression against a sexually receptive female, vom Saal and Bronson (1978) found that a higher proportion of 2M CF-1 females fought compared to 0M females. However, there were no differences between 0M and 2M females in the latency or frequency of attacks (vom Saal and Bronson, 1978).

Although the expression of these behaviors are thought to occur through the activation of neural mechanisms responsive to testosterone, the previous studies have not examined potential differences in the sensitivity of these neural regions to testosterone. Sensitivity is a measure of the responsiveness of neural tissues to the activational effects of the steroid. Therefore increased sensitivity to testosterone would be indicated by a shorter duration of testosterone treatment required to induce the dependent behavior. Within this definitional framework, weekly tests and measures of the total proportion of females fighting minimizes the likelihood of detecting differences in sensitivity. In addition, an argument for the masculinization of neural substrates regulating the expression of aggressive behavior in females due to uterine position is not supported by evidence of aggression against females. Intermale aggression, reportedly organized by perinatal testosterone exposure, must be tested by using a male conspecific. This androgen dependent behavior is typically expressed against other males and its display is attenuated when males are tested against a female conspecific (Edwards and Rowe, 1974; Brain, Haug, and Kamis, 1983)

A more appropriately designed study examining the effects of uterine position on neural sensitivity to testosterone was conducted by Gandelman, vom Saal, and Reinisch (1977). These investigators examined the duration of testosterone treatment required to activate aggression among Rockland-Swiss females from different uterine positions using an olfactory bulbectomized male as the stimulus animal. It was found that 2M females required approximately 15 days of testosterone treatment to activate aggression while 0M females required approximately 25 days of treatment. These results alone suggest that uterine position may cause differential sensitivity in neural mechanisms regulating intermale aggression. However, the generalizability

of these findings is unknown since they are currently limited to a single mouse strain.

Close examination of the available literature suggests that findings of uterine position effects have not been consistent across rodent strains and species or across behaviors. The current studies, therefore, were primarily designed to extend findings of uterine position effects on sensitivity to the aggression-promoting property of testosterone in CK and CF-1 female mice. Aggression was selected as the behavioral system as opposed to mounting behavior because, as discussed earlier, tests for aggression could be conducted every other day instead of once a week, permitting a better measure of neural sensitivity. The behavioral data was then followed by biochemical analyses of the concentration of androgen binding in the hypothalamus of CF-1 females from different uterine positions. Since the expression of androgen-dependent behaviors, such as aggression, is dependent on androgen binding in neural regions regulating the expression of that behavior, this measure provided an additional index for measuring differential androgen sensitivity in female mice. Additional physiological measures included ano-genital distance to body weight ratio at birth, 21 days, and 60 days of age; onset of puberty as assessed by daily examination of vaginal smears; and subsequent estrus cycle lengths.

## GENERAL METHODS

### Animals

All housing and experimental conditions met Federal guidelines for the care and treatment of animals. CK female mice were derived from a C57BL/6J x AKR/J cross and CF-1 female mice were bred from parent stock purchased from the Charles River Breeding Farm (Wilmington, MA). Mice were housed in 28 x 18 x 13 cm polypropylene cages on a bedding of wood

chips with food and water provided in excess. Rooms were maintained at 23 ± 2° C on a 12 hour light/dark cycle, with lights on at 0800 hours. Each room contained both male and female mice.

Mating and Delivery

Breeding began when the female mice were 60 days of age. Two male mice were introduced for several hours on a daily basis into the home cage of four females. Females with visible plugs were separated and individually housed. Females that were visibly pregnant during this mating period but whose vaginal plugs were not observed were also individually housed and used as foster mothers once they delivered.

On the evening of the 18th day of gestation the offspring were delivered by cesarean section. The pregnant female was sacrificed by cervical dislocation, the uterus was exposed by a midline incision and the individual fetuses carefully removed (maintaining their relative position), placed on a heating pad and cleansed with fresh water. The offspring were sexed and the uterine position of the female offspring relative to male littermates was established. For purposes of clarity, fetuses located toward the cervical end of the uterine horn will be referred to as caudal, while fetuses located toward the ovarian end of the uterine horn will be referred to as rostral. Female fetuses were classified as 0M if they were contiguous to two females, if they were located at the ovarian end of the uterine horns with a single contiguous caudal female, or if they were located at the cervical end of the horn with a rostral female and another female at the cervical end of the adjacent uterine horn. They were classified as 2M if they were contiguous to two males, 1C if they were between a caudal male and a rostral female, and 1R if they were between a rostral male and a caudal female. The 1R and 1C females were also collectively referred to as 1M. Other than the placement of the 0M females

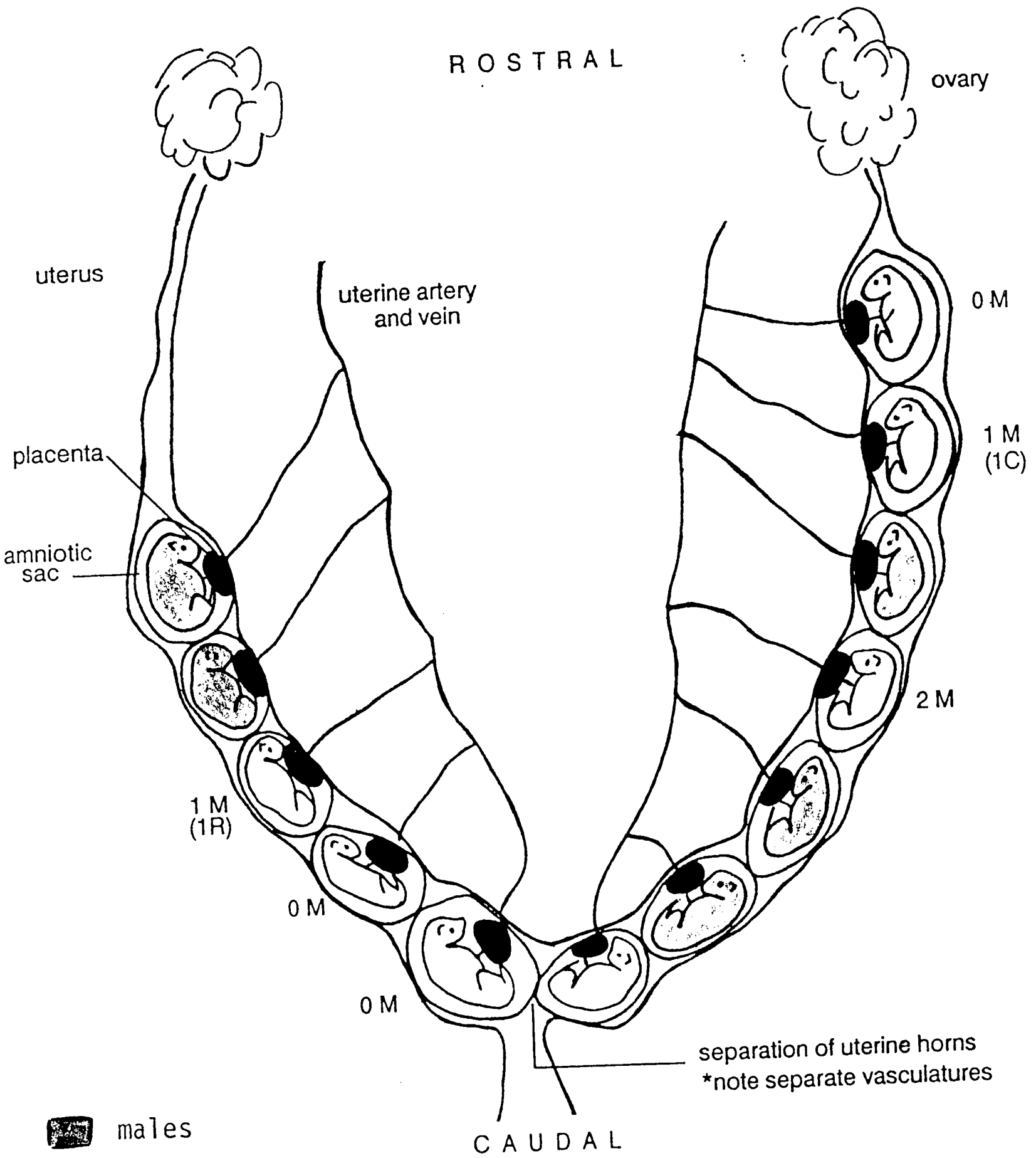
discussed above, all other females located at the cervical end of the uterine horn were excluded due to discrepencies between the contiguity and caudal classification schemes. The two uterine horns at the cervical end are in close contact, so that according to the contiguity hypothesis, diffusion of hormones between the adjacent amniotic sacs at each of these ends is possible. However, the two horns have separate vasculatures, so according to the caudal hypothesis there would be no hormone interaction between fetuses at the cervical ends. Figure 1 illustrates the uterine positions and the uterine vasculature.

Upon delivery, females were fostered to mothers that had delivered within the preceding 48 hours and whose young had been removed. In Experiment 1, the females were marked according to their uterine position by toe clipping, however, in Experiment 2 the females were fostered in like uterine position litters to avoid marking and males were added to maintain a constant litter size of six.

Surgery and Aggression Tests

In adulthood females were ovariectomized under nembutal anesthesia and implanted with 10 mm silastic capsules containing 5 mg testosterone/.02 cc oil. They were individually housed and tested for the display of aggressive behavior beginning 10 to 14 days later. Testing was conducted every other day for twenty days, for a total of 10 behavioral tests. Each test session began when an olfactory bulbectomized CF-1 male was introduced into the female's home cage for a ten minute period. The number of attacks by the female against the male were tallied. If the female reached five attacks she was designated a fighter, testing was terminated, and she was not tested again.

Figure 1: Classification of female mouse fetuses based on position relative to males during uterine development.



## Experiment 1

This study was designed to extend the findings by Gandelman, vom Saal, and Reinisch (1977) that described differences in T sensitivity among Rockland-Swiss female mice from different uterine positions. Sensitivity to the aggression-promoting property of testosterone was examined in CF-1 and two generations of CK adult female mice.

### METHODS

The uterine position of two generations of CK female mice (CKF1 and CKF2) and one generation of CF-1 female mice were established using the procedure outlined in the General Methods. The females were weaned at 21 days of age and group housed with same sex littermates. Surgery, testosterone treatment, and behavioral testing for aggressive behavior is described in the General Methods.

The females from known uterine positions were implanted with testosterone capsules at 9 to 11 months of age and testing for aggression began 14 days later.

### RESULTS

In an attempt to pool the data across the two strains preliminary analyses were conducted between CF-1 and CK female mice as well as between the two CK generations examining possible differences in aggressive behavior. Because no differences were found between CKF1 and CKF2 females in the proportion of females which fought ($X^2_{(1)} = 0.11$, $p>.05$) or in the number of days of testosterone treatment required to activate aggression ($t(32) = 1.98$, $p>.05$), all subsequent analyses for CK females combined the data from these two generations. Comparisons across strains, however, found that a higher proportion of CF-1 females fought (($X^2_{(1)} = 11.74$, $p<.05$), and that they also required a shorter period of testosterone treatment before aggression was

activated ($t(89) = 2.37$, $p<.05$). These results are shown in Table 1. Based on these findings, each strain was analyzed independently.

**Table 1:** Experiment 1 - Aggressive behavior exhibited by CK and CF-1 female mice in response to testosterone implants.

| Genotype | Proportion Fighting [a] | Latency to Fight (days) [b] ($x \pm SE_m$) |
|---|---|---|
| CK | 34/56 61% | 18.46 ± 0.93 |
| CF-1 | 57/65 88% | 16.00 ± 0.58 |

[a] sign. difference between strains: $X^2_{(1)} = 3.84$, $p<.05$

[b] sign. difference between strains: $t(89) = 2.37$, $p<.05$

The aggressive behavior displayed by CF-1 females from each of the three uterine positions is summarized in Table 2. A comparison between the three groups of females in the proportion of fighting ($X^2_{(2)} = 3.52$, $p>.05$) and the number of days of treatment required to activate aggression ($F(2,54) = 0.15$, $p>.05$) found no differences.

**Table 2:** Experiment 1 - Aggressive behavior exhibited by 0M, 1M, and 2M CF-1 female mice in response to testosterone implants.

| Uterine Position | Proportion Fighting [a] | Latency to Fight (days) [b] ($x \pm SE_m$) |
|---|---|---|
| 0M | 15/18 83% | 16.11 ± 1.10 |
| 1M | 33/35 94% | 16.36 ± 0.37 |
| 2M | 9/12 75% | 16.67 ± 0.69 |

[a] no sign. difference among uterine positions: $X^2_{(2)} = 3.52$, $p>.05$

[b] no sign. difference among uterine positions: $F(2,54) = 0.15$, $p>.05$

Table 3: Experiment 1 - Aggressive behavior exhibited by 0M, 1M, and 2M CK female mice in response to testosterone implants.

| Uterine Position | Proportion Fighting [a] | Latency to Fight (days) [b] ($x \pm SE_m$) |
|---|---|---|
| 0M | 12/16 78% | 16.67 ± 1.27 [c] |
| 1M | 15/28 53% | 21.33 ± 1.53 [d] |
| 2M | 7/11 63% | 15.43 ± 0.67 [c] |

[a] no sign. difference among uterine positions: $X^2_{(2)} = 1.99$, $p>.05$

[b] sign. difference among uterine positions: $F(2,31) = 4.50$, $p<.05$

[c] 0M and 2M not sign. different: Scheffe's $t = 0.45$, $p>.05$

[d] 1M sign. different from 0M and 2M: Scheffe's $t = 3.89$, $p<.05$

Data from the CK females is shown in Table 3. The proportion of 0M, 1M, and 2M females that fought did not differ ($X^2_{(2)} = 1.99$, $p>.05$). However, there was a difference in the number of days of testosterone treatment required to activate aggression ($F(2,31) = 4.50$, $p<.05$). As can be seen in Table 3, the 1M females required a longer mean treatment duration. Post-hoc comparisons using Scheffe's test showed that while the 2M and 0M female groups did not differ from each other, their mean treatment durations were shorter in comparison to the 1M group.

The 1M females were then divided into 1R and 1C females, based on their prenatal contiguity to male fetuses, in an attempt to examine the blood flow hypothesis outlined by Meisel and Ward (1981). Table 4 shows the data. T-tests comparing the number of days of testosterone treatment required to induce fighting found no difference between 1R and 1C females in either the CF-1 strain ($t(30) = .95$, $p>.05$) or the CK strain ($t(13) = 1.92$, $p>.05$).

Table 4: Experiment 1 - Number of days of T treatment required to activate aggression in 1M female mice - Comparison between females contiguous to either a rostral or caudal male (1R vs. 1C).

| Uterine Position | CF-1 [a] ($x \pm SE_m$) | CK [b] ($x \pm SE_m$) |
|---|---|---|
| 1R | 15.85 ± .39 | 17.60 ± 1.91 |
| 1C | 16.53 ± .53 | 23.20 ± 1.81 |

[a] no sign. difference among uterine positions: $t(30) = 0.95$, $p>.05$

[b] no sign. difference among uterine positions: $t(13) = 1.92$, $p>.05$

## DISCUSSION

The effects of uterine position on testosterone sensitivity found in adult Rockland-Swiss females (Gandelman, vom Saal, and Reinisch, 1977) could not be extended to include CK and CF-1 females. The present results found no differences in the number of days of treatment required to induce aggressive attack in CF-1 females. Further, while an overall difference was found between 0M, 1M, and 2M CK females, it was not consistent with the uterine position model, because 1M females required a significantly longer duration of T exposure than either 0M or 2M females to activate aggression. The fact that there were no differences in the proportion of females across uterine position or strain in response to testosterone treatment is not surprising given the long duration of testosterone exposure and testing.

However, these findings should be viewed cautiously because the animals were fairly old at the time of testing and had undergone previous steroid treatments and behavioral testing for ultrasound vocalizations and preference behavior. Studies examining changes in reproductive cycles in

aging female mice suggest that neural regions regulating these cycles are no longer sensitive to the feedback control of ovarian steroids (Gray and Wexler, 1980; Gray, Tennent, Smith, and Davidson, 1980; Kohama, Anderson, Osterburg, May, and Finch, 1989). It is possible, therefore, that the similarity in responsiveness observed in the current study was due to the age of the females, so that differential responsiveness to testosterone may be found in young CF-1 females from different uterine positions.

**Experiment 2**

Because findings from the first study found no differences in aged CF-1 and CK female mice as a function of their uterine position, it was of interest to further investigate potential differences among young adult female mice from known uterine positions. The second experiment examined the effects of uterine position on sensitivity to aggression-promoting property of testosterone in CF-1 female mice. CF-1 females from known uterine position were implanted with testosterone at 60 days of age and tested for aggression beginning at 70 days of age. Morphological and physiological measures were included in an attempt to replicate previous findings of uterine position effects in this strain and provide an additional data base from which to interpret the behavioral findings.

## METHODS

CF-1 female mice from known uterine position were generated using the general methodology for mating and delivery outlined above. In addition, the newborns were weighed to the nearest 0.001 g and ano-genital distance was measured to the nearest 0.01 mm with calipers and a dissecting microscope. The ano-genital distance was measured from the base of the phallus to the base of the anus.

At 21 days of age, the offspring were weaned. Females were housed with littermates of the same uterine position in a room with additional male and female mice. Ano-genital distance and body weight were again measured. Following weaning, the females were observed daily for the age of vaginal opening.

At 35 days of age, vaginal smears were taken for 15 consecutive days. Approximately 100 ul of physiological saline (0.9%) was introduced into the vagina and immediately aspirated with a glass dropper. The saline was then applied to a glass slide, followed by a drop of Coomassie blue (50:50 Coomasie blue with $H_2O$, v:v). The slide was then dipped into a bath of fresh water and allowed to dry overnight. The slides were observed under a microscope and estrus phase was determined based on the predominant cell type. (Pictures taken of random slides representative of the different cell types and their corresponding estrus phases are presented in Plates 1-4). One complete estrus cycle was the number of days between fully cornified vaginal smears, with Day 1 of the cycle being the first observation of a cornified smear.

Between 50 and 60 days of age, ano-genital distance and body weight were measured for a third time. The females were then ovariectomized under nembutal anesthesia supplemented with ether and implanted with 10 mm silastic capsules containing 5 mg testosterone/.02 cc oil. Approximately one-third of the females from each uterine position served as a control group and received 10 mm silastic capsules containing oil vehicle only. The females were individually housed and behavioral testing for aggressive behavior began 10 days later, following the methodology described in General Methods. At the conclusion of the behavioral testing the silastic implants were removed.

When the females were approximately 120 days of age, four females from each uterine position were randomly chosen and injected with 300 ug testosterone/.02 cc oil for eight consecutive days. Fourty-eight hours after the final injections, the females were sacrificed and the brains were removed and assayed for androgen binding. Two experimental replicates were performed for T-treated females.

Androgen Binding Assay

The females were sacrificed by cervical dislocation and the brains were rapidly removed and blocked on ice. The hypothalamus, preoptic area, and septum were removed in a single block. The section was bordered posteriorly by the mammillary bodies, laterally by the hypothalamic sulci, and anteriorly by a cut approximately 1.5 mm anterior to the optic chiasm. The septum was included by making a diagonal cut between the posterior and anterior borders of the section to a final depth of approximately 3 mm rostral to the preoptic area.

All subsequent steps were carried out at 0-4° C. Brain sections (4 sections/ml from each uterine position) were placed in a teflon-glass homogenizer containing 1 ml TEDGM buffer (10 mM Tris HCl, 1.5 mM dithiothreitol, 10 mM sodium molybdate, 1.5 mM EDTA, 20% glycerol v/v, pH 7.4 at 0° C). They were homogenized by 20 strokes followed by a 1 ml wash and 8 additional strokes. The homogenate and wash were centrifuged in a fixed angle rotor for 10 minutes at 800 x g. The supernatant was then centrifuged in a Beckman SW 50.1 rotor for 1 hour at 100,000 x g. To measure total binding from each uterine position, 200 ul of the high speed supernatant containing solubalized receptors was incubated in 12 x 75 glass tubes pretreated with 0.1% BSA containing a 5 nM dose of radiolabeled dihydrotestosterone ([3H]DHT, specific activity 156.6 Ci/mmol, New England

Nuclear). Parallel tubes containing 200 ul cytosol, labeled DHT and a 100x excess of unlabeled hormone were used to determine nonspecific binding. Final volume of each tube was 250 ul: 200 ul cytosol, 10 ul TEDGM buffer with or without a 100x excess of unlabeled DHT, and 40 ul 5 nM [3H]DHT in TEDGM. Internal duplicates were run for both total and nonspecific binding tubes for each condition.

Previous findings (data not shown) indicated that androgen receptor binding reached equilibrium after 8 hours of incubation. Therefore, incubations in the current study were terminated after 8 hours with 250 ul of a hydroxylapatite (HAP) suspension (50/50 HAP with TE, v/v). The tubes were vortexed immediately for 10 seconds, then for 10 seconds every 10 minutes for 30 minutes. The incubates were then centrifuged in a swinging bucket rotor for 4 minutes at 800 x g. The supernatant was removed by aspiration, and the HAP pellet was washed 5 times with 2 ml TE containing 1% Tween-80 (polyoxyethylenesorbitan monooleate; Sigma Chemical Co.). Each wash was followed by a 3 minute centrifugation at 800x g.

After the final wash, the walls of the incubation tubes were wiped with ethanol and dried with cotton swabs. The washed pellet was extracted with 1 ml absolute ethanol and centrifuged at 800 x g for 3 minutes. 900 ul aliquots were taken and placed in 20 ml scintillation vials. The volume of absolute ethanol was brought back to 1 ml and the procedure was repeated. Ten ml of Ecoscint floor was added to each scintillation vial. Radioactivity of each sample was measured in a Beckman LS-8100 scintillation counter.

Specific [3H]DHT binding levels were determined by subtracting nonspecific from total binding. Protein concentrations were determined using a Bio-Rad Dye Binding Reagent Kit and all data were normalized by converting to a per mg protein basis.

## RESULTS

Comparisons of ano-genital distance to body weight ratio are shown in Table 5.

**Table 5**: Experiment 2 - Ano-genital distance to body weight ratio in 0M, 1M , and 2M CF-1 female mice.

| Uterine Position | Ano-Genital Distance:Body Weight (mm:g) | | |
|---|---|---|---|
| | Birth [a] ($x \pm SE_m$) | Weaning [b] ($x \pm SE_m$) | 60 Days [c] ($x \pm SE_m$) |
| 0M | 0.78 ± .02 | 0.26 ± .05 | 0.17 ± .003 |
| 1M | 0.79 ± .01 | 0.25 ± .004 | 0.18 ± .003 |
| 2M | 0.79 ± .02 | 0.24 ± .005 | 0.17 ± .003 |

[a] no sign. difference among uterine positions: $F(2,178)= 0.12$, $p>.05$

[b] sign. difference among uterine positions: $F(2,91) = 3.10$, $p<.05$

[c] no sign. difference among uterine positions: $F(2,78) = 0.74$, $p>.05$

The data for ano-genital: body weight ratios are derived from all female pups of known uterine position carried to term by the mother. By the time of weaning (Day 21) the number of females included in the analysis was reduced due to a 24% (13/53) rejection rate of the litters by the foster mothers. No differences were found between 0M, 1M, and 2M females at birth ($F(2,178) = 0.115$, $p>.05$), or at 60 days of age ($F(2,78) = 0.74$, $p>.05$). A difference was found at weaning ($F(2,91) = 3.10$, $p<.05$), with 2M females having a smaller ratio than 0M females.

Analyses examining the effects of uterine position on the age of vaginal opening and estros cycle lengths again found no differences between 0M, 1M,

and 2M females. The number of contiguous males during prenatal development did not alter the age of vaginal opening ($F(2,91) = .45$, $p>.05$) or the number of days in an estros cycle ($F(2,70) = 2.96$, $p>.05$) exhibited by the females. Table 6 shows the data. Vaginal opening occurred at approximately 28 days of age with subsequent estros cycles lasting from 5 to 5.5 days.

**Table 6:** Experiment 2 - Age of vaginal opening and estrus cycle lengths exhibited by 0M, 1M, and 2M CF-1 female mice.

| Uterine Position | Vaginal Opening (days) [a] ($x \pm SE_m$) | Estrus Cycle Length (days) [b] ($x \pm SE_m$) |
|---|---|---|
| 0M | 28.32 ± 0.43 | 5.17 ± 0.11 |
| 1M | 28.37 ± 0.33 | 5.59 ± 0.12 |
| 2M | 27.84 ± 0.50 | 5.49 ± 0.16 |

[a] no sign. difference among uterine positions: $F(2,91) = .45$, $p>.05$

[b] no sign. difference among uterine positions: $F(2,70) = 2.96$, $p>.05$

Differences were found between the treatment and control groups. None of the females given implants containing oil vehicle displayed aggressive behavior against the stimulus male. In contrast, approximately 85% of the females receiving T filled implants fought, requiring an average of about 14 days of exposure to activate the behavior. However, a comparison among uterine positions found no differences between 0M, 1M, and 2M females in the proportion of animals which fought in response to testosterone ($X^2_{(2)} = 0.25$, $p>.05$) or in the number of days of testosterone treatment required to activate aggression ($F(2,47) = 0.06$, $p>.05$). The data is presented in Table 7.

**Table 7:** Experiment 2 - Aggressive behavior exhibited by 0M, 1M, and 2M CF-1 female mice following testosterone treatment.

| Uterine Position | Proportion Fighting [a] | Latency to Fight (days) [b] ($\bar{x} \pm SE_m$) |
|---|---|---|
| 0M | 18/21 86% | 13.67 ± 0.80 |
| 1M | 18/22 82% | 14.00 ± 0.65 |
| 2M | 14/16 87% | 13.86 ± 0.57 |

[a] no sign. difference among uterine positions: $X^2_{(2)} = 0.25$, $p>.05$

[b] no sign. difference among uterine positions: $F(2,47) = 0.06$, $p>.05$

The data was then analyzed with respect to the caudal hypothesis proposed by Meisel and Ward (1981). The 1M females were divided into 1R and 1C females based on their uterine position, and compared for differences on the measures discussed above, including ano-genital distance to body weight ratio at birth, 21 and 60 days, age of vaginal opening, estrus cycle length, and display of aggressive behavior in response to testosterone treatment. No differences were found between 1R and 1C females on any of these measures. Table 8 shows the data.

Binding levels of [3H]DHT in the hypothalamic-preoptic-septal region of 0M, 1M, and 2M females following eight days of testosterone treatment are shown in Table 9. Statistical analyses were not conducted due to the small n. Androgen binding for each uterine position was consistent within and between experiments. There were no differences between 0M and 2M females in induced binding, although 1M females had higher mean binding than both 0M and 2M females.

TABLE 8: Experiment 2 - Comparison of 1M CF-1 female mice - females contiguous to either a rostral or caudal male during uterine development (1R vs. 1C).

| Uterine Position | AG:BW (mm:g) Birth [a] ($\bar{x}$) | Wean [b] ($\bar{x}$) | 60 dys [c] ($\bar{x}$) | Cyclicity (days) VO [d] ($\bar{x}$) | Estrous [e] ($\bar{x}$) | Aggression Latency to Fight (dys) [f] ($\bar{x}$) |
|---|---|---|---|---|---|---|
| 1C | 0.78 | 0.26 | 0.17 | 28.64 | 5.56 | 15.00 |
| 1R | 0.81 | 0.25 | 0.18 | 26.50 | 5.42 | 13.11 |

[a] $t(78) = 0.89$

[b] $t(48) = 0.81$

[c] $t(41) = 1.31$

[d] $t(17) = 2.44$*

[e] $t(23) = 0.59$

[f] $t(11) = 1.26$

* significant difference, $p<.05$

**Table 9**: Experiment 2 - Androgen binding in the hypothalamic-preoptic-septal region of 0M, 1M, and 2M CF-1 female mice following testosterone treatment.

| Uterine Position | Bound (dpm/mg protein) * |
|---|---|
| 0M | 1235 |
| 1M | 1404 |
| 2M | 1227 |

* Results shown are mean dpm/mg protein for two independent replications. Each replication had internal duplicates.

## DISCUSSION

Despite earlier evidence from other research groups for differential degrees of masculinization in female mice due the number of contiguous males present during prenatal development, results from this study did not support such a model. Differential lengths of ano-genital distance were reported in CF-1 (vom Saal and Bronson, 1978) and Rockland-Swiss female mice (Gandelman, vom Saal, and Reinsich, 1977), as well as Sprague-Dawley female rats (Clemens, 1974; Meisel and Ward, 1981; Richmond and Sachs, 1984). Although not all the studies accounted for the effects of body weight on this morphological measure, 2M females consistently exhibited longer ano-genital distances than their 0M littermates at birth. Further, vom Saal and Bronson (1978) reported that this difference between 0M and 2M CF-1 females remained in adulthood. The current study found no differences based on uterine position in ano-genital distance to body weight ratios at birth in CF-1 female mice. By weaning, where a significant difference in ano-genital distance to body weight was found, 0M females had a larger ratio than 2M

females, a finding not consistent with the uterine position model. At 60 days of age there was again no difference between 0M, 1M, and 2M females on the ratio of ano-genital distance to body weight.

A possible explanation for the lack of anogenital distance to body weight ratio differences is that the effects of prenatal exposure to testosterone secreted by male littermates had a more generalized effect on morphology, causing 2M females to be heavier and have longer ano-genital distances than 0M or 1M females. Direct comparisons of these parameters, however, does not support this concept. More specifically, comparisons of body weight among females from the three positions revealed significant differences at birth ($F(2, 178) = 3.34$, $p<.05$), weaning ($F(2,91) = 6.72$, $p<.05$), and adulthood ($F(2, 78) = 3.14$). However, post hoc comparisons revealed that the effect was due to 0M females being consistently heavier than 1M females; body weight did not significantly differ at any age between 0M and 2M females. In addition, comparisons of ano-genital distance between the three uterine positions also found no significant differences at birth or 60 days of age. A significant difference was found at weaning ($F(2,91) = 6.42$, $p<.05$), but again, post-hoc comparisons showed that 0M females had a longer ano-genital distance than 1M females (Scheffe's $t = 6.41$, $p<.05$). Therefore, the uterine position of CF-1 female mice with respect to males did not produce any systematic morphological changes normally associated with differences in T exposure.

Uterine position effects on variations in the onset of sexual maturity also were not supported. Studies examining age of vaginal opening as an index of sexual maturity in rats and gerbils found that 0M females exhibited vaginal opening at an earlier age than 2M females (McDermott, Gandelman, and Reinisch, 1978; Clark and Galef, 1988). However, in the current study differences in the age of vaginal opening in CF-1 female mice were not found.

Strain variations may contribute to these differential effects, especially since sex ratio in litter composition of female gerbils is highly correlated with whether or not the dam was early or late maturing. Early maturing dams tend to produce large female:male ratio litters with female offspring predominantly early maturing, while late maturing dams tend to produce small largely male:female ratio litters with female offspring predominantly late maturing (Clark, Spencer, and Galef, 1986; Clark and Galef, 1988).

An examination of estros cycle lengths in adult CF-1 female mice also was inconsistent with previous findings. It has been reported that adult 2M CF-1 females have longer estrus cycles than 0M females when group housed in the presence of a male (vom Saal and Bronson, 1980; vom Saal, Pryor, and Bronson, 1981). In contrast to these findings, the present study found no differences between 0M and 2M females on estrus cycle length. However, these findings may not have been representative of normal adult cycles since daily vaginal smears began at an early age and females were not housed with a male. vom Saal reported that the duration of the first estrus cycle occurring around 32 days of age is longer in 0M females when compared to 2M females, and these early cycles are longer than subsequent cycles. Early cycles ranged from 6.5 days in 2M females to 8 days in 0M females (vom Saal, Pryor, and Bronson, 1981), while adult cycles ranged from 5.3 days in 0M females to 6.4 days in 2M females (vom Saal and Bronson, 1980). The present study began vaginal smearing at 35 days of age and recorded up to three complete cycles. Using animals in which at least two complete cycles were measured, no difference in the length of these cycles was found in the current study ($t(76) = 1.21$, n.s.). In addition the cycle lengths fall with the range reported for adult CF-1 females, with a mean of approximately 5.30 days. This suggests that the CF-1 females in the current study had completed their first estrus cycle by the

time vaginal smears began and displayed adult patterns of ovulation. Based on this evidence, unlike the difference reported by vom Saal and his collegues for adult CF-1 female mice (vom Saal and Bronson, 1980), 2M females did not display longer estrus cycles than 0M females.

Another possibility which may be raised to account for the disrepency in vom Saal's findings of uterine position effects on adult estrus cycles and the current findings is the housing condition of the females. 2M females have reportedly displayed longer estrus cycles than 0M females when group housed in the presence of a male, while the present study reported no effects of uterine position on estrus cycle lengths when group housed in the absense of a male. It has been reported that when females are group housed in the absence of a male cycle lengths are increased to approximately 10 days (vom Saal and Bronson, 1980) and become irregular (Vandenbergh, 1974; Bronson, 1979). However, this study found that estrus occurred in regular 5.50 day cycles. It is likely that CF-1 males present in the room but caged separately from the females were sufficient to expose the females to the necessary pheromonal cues required to regulate their estrus cycles to similar patterns displayed by adult females group housed with a male.

Along with investigating possible physiological variations in CF-1 females due to uterine contiguity to males, this study also examined variations in testosterone sensitivity among the females. Previous reports found that a rank order of androgen sensitivity was established in Rockland-Swiss female mice due to the number of contiguous males during prenatal development (Gandelman, vom Saal, and Reinisch, 1977). Thus 2M females required the shortest duration of androgen exposure to activate aggression against a male conspecific, 1M females an intermediate duration, and 0M females required the longest duration of exposure. However, the effects of uterine position on

aggressive behavior appear to be genetically constrained since these findings were not extended to CF-1 females. CF-1 females behaviorally responded much sooner to testosterone treatment than Rockland-Swiss females, fighting after approximately 14 days of exposure while aggression was not activated in Rockland-Swiss females until at least 15 days of testosterone exposure, with the least responsive group of females requiring up to 25 days of treatment.

These behavioral findings suggest that for CF-1 females, uterine contiguity to males does not alter the sensitivity of neural substrates that mediate the expression of aggressive behavior. This premise was consistent with biochemical data which found no differences in induced androgen binding in neural regions subserving aggressive behavior in mice. It is possible, however, that differences in basal levels of androgen binding existed among the groups but were not detected due to testosterone treatment. However, even if such differences exist, they do not affect sensitivity to the behavior-promoting effect of exogenously administered testosterone during adulthood.

It is possible that full masculinization of neural substrates for aggression may be a product of postnatal hormone exposure. Postnatal testosterone treatment can masculinizes neural regions regulating aggressive behavior (Edwards, 1968; Bronson and Desjardins, 1970; Whitsett, Bronson, Peters, and Hamilton, 1972; Barkley and Goldman, 1977) and thereby increase subsequent sensitivity to the aggression-promoting property of testosterone in adulthood. Therefore if full masculinization of aggressive behavior does not occur until postnatal life, prenatal variation in testosterone exposure as presented in the uterine position phenomenon would have little effect on generating individual differences in the eventual expression of androgen-dependent aggressive behavior.

# GENERAL DISCUSSION

The uterine position phenomenon has been proposed as a mechanism to explain individual variation among female rodents. This mechanism was based on early findings in female rats and suggested that testosterone secreted by males during uterine development diffused across the amniotic sacs and entered the blood stream of contiguous littermates (Clemens, 1974). This prenatal testosterone exposure in female littermates reportedly caused slight alterations in the sensitivity of neural and peripheral tissues to androgen (Clemens, Gladue, and Coniglio, 1978) and was supported by behavioral and physiological findings in rats and mice (McKermott, Gandelman, and Reinisch, 1978; Meisel and Ward, 1981; Quadagno, McQuitty, McKee, Kowlliker, Wolfe, and Johnson, 1987) . For instance, females that developed between two males in utero (2M) had longer ano-genital distances, displayed longer estrus cycle lengths, and enhanced sensitivity to testosterone when compared with females that developed between two females (0M) (vom Saal and Bronson, 1978; vom Saal and Bronson, 1980; vom Saal, 1983).

Establishing intrauterine position as a generalized phenomenon, however, is difficult based on the current nature of the research evidence. Several shortcomings are evident. The first regards a tendency for the findings to be isolated to a single species or strain. For instance, effects of uterine position on estrus cycle lengths have been reported only in CF-1 female mice and were largely dependent on housing conditions. Adult 2M CF-1 females expressed longer cycle lengths only when group housed in the presence of a male or individually housed in the absence of a male (vom Saal, 1981). Further, effects of enhanced sensitivity to the aggression-promoting property of testosterone were reported in 2M Rockland-Swiss female mice but not in other mouse strains or rodent species (Gandelman, vom Saal, and

Reinisch, 1977). A second shortcoming is that the uterine position effects only alter the expression of those behaviors and physiological parameters which differentiate prenatally. In species with relatively short gestation periods, such as hamsters and mice, sexual differentiation continues postnatally and therefore the range of parameters which may be affected by this model become limited. For instance, uterine position effects on the expression of mounting behavior following ovariectomy and androgen treatment are more conclusive for rats than mice (Clemens, Gladue, and Coniglio, 1978; Meisel and Ward, 1981) . The data from mice have produced variable results with some reports indicating an increased frequency of mounting behavior in 2M females over 0M females (Rines and vom Saal, 1984; Quadagno et a., 1987), while other reports found no differences between 0M and 2M females (vom Saal and Bronson, 1978; Gandelman and Kozak, 1988). A final issue, although not in itself a shortcoming, concerns an alternative theory for the reported uterine position effects. This theory suggests that testosterone secreted by male fetuses diffuses across the uterine arteries and veins rather than the amniotic sacs (Meisel and Ward, 1981). While these models are not mutually exclusive, there has been no direct evidence to support either the blood flow hypothesis or the contiguity hypothesis for the reported differences in female rodents due to uterine position effects.

The current studies attempted to replicate and extend some of the reported findings, applying both the contiguity and blood flow models. CF-1 and CK female mice were bred and their uterine position with respect to male littermates was established. The first experiment focused on differences in sensitivity to the aggression-promoting property of testosterone based on uterine position and strain in adult CF-1 and CK females. The second

experiment examined uterine position effects on ano-genital distance at birth, 21 days, and 60 days of age; age of vaginal opening; estrus cycle lengths in adulthood; sensitivity to the aggression-promoting property of testosterone; and androgen binding concentrations in hypothalamic tissue in CF-1 females. Comparisons between 0M and 2M females yielded no significant findings on any of the measures in either experiment 1 or 2. Differences were found between CF-1 and CK females in their sensitivity to testosterone. A higher proportion of CF-1 females attacked a stimulus male in response to androgen treatment. These CF-1 females also required a shorter duration of androgen exposure to activate an aggressive attack than CK females.

In general, the findings from these studies do not support uterine position as a mechanism for generating variation among CF-1 and CK female mice. While this does not refute the utility of the uterine position phenomenon to explain individual variation it does limit its general application. Many of the gaps in the literature on uterine position effects may reflect limitations in this model imposed by species and strain variations, specifically differences in the critical periods of sexual differentiation and sensitivity to testosterone.

# REFERENCES


Barkley, M. S. and Goldman, B. D. The effects of castration and silastic implants of testosterone on intermale aggression in the mouse. Hormones and Behavior, 9, 32-48 (1977).

Beach, F. A. Hormonal factors controlling the differentiation, development and display of compulatory behavior in the ramstergig and related species. In Biopsychology of Development (Eds. E. Tobach, L. R. Aronson, and E. Share) Academic Press, New York, 1971.

Brain, P.F., Haug, M., and Kamis, A. Hormones and different tests for aggression with particular reference to the effects of testosterone metabolites. In Hormones and Behavior in Higher Vertebrates (Eds. J. Balthazart, E. Prove, and R. Gilles) Springer-Verlag, Berlin, 1983.

Bronson, F. H. and Desjardins, C. Neonatal androgen administration and adult aggressiveness in female mice. General and Comparative Endocrinology, 15, 320-325 (1970).

Clark, M. M., Spencer, C. A., and Galef Jr., B. G. Reproductive life history correlates of early and late maturation in female Mongolian gerbils (Meriones unguiculatus). Animal Behavior, 34, 551-560 (1986).

Clark, M. M. and Galef, Jr., B. G. Effects of uterine position on rate of sexual development in female mongolian gerbils. Physiology and Behavior, 42, 15-18 (1988).

Clemens, L. G. and Coniglio, L. Influence of prenatal litter composition on mounting behavior of female rats. American Zoologist, 11, 617-618 (1971).

Clemens, L. G. Neurohormonal control of male sexual behavior. In Reproductive Behavior (Eds. W. Montagna and W. A. Sadler) Plenum Press, New York, 1974.

Clemens, L. G., Gladue, B. A., and Coniglio, L. P. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. Hormones and Behavior, 10, 40-53 (1978).

Coniglio, L. P., Paup, D. C., and Clemens, L. G. Hormonal factors controlling the development of sexual behavior in the male golden hamster. Physiology and Behavior, 10, 1087-1094 (1973).

Drickamer, L. C. Delay of sexual maturation in female mice by exposure to grouped females or urine of grouped females. Journal of Reproduction and Fertility, 51, 77-81 (1977).

Edwards, D. A. Mice: fighting by neonatally androgenized females. Science, 161, 1027-1028 (1968).

Edwards, D. A. and Rowe, F. A. Neural and endocrine control of aggressive behavior. In Hormonal Correlates of Behavior (Eds. B. Eleftheriou and R. Sprott) vol. 1, Plenum Press, New York, 1974.

Gandelman, R., vom Saal, F. S., and Reinisch, J. M. Contiguity to male foetuses affects morphology and behavior of female mice. Nature, 266, 722-724 (1977).

Gandelman, R. Uterine position and the activation of male sexual activity in testosterone propionate-treated female guinea pigs. Hormones and Behavior, 20, 287-293 (1986).

Gandelman, R. and Kozak, M. Neonatal testosterone administration, but not in utero contiguity to males, augments the display of male sexual behavior in testosterone-treated adult female mice. Physiology and Behavior, 42, 453-456 (1988).

Gray, G. D., Tennent, B., Smith, E. R., and Davidson, J. M. Luteinizing hormone regulation and sexual behavior in middle-aged female rats. Endocrinology, 107, 187-194 (1980).

Gray, G. D., and Wexler, B. C. Estrogen and testosterone sensitivity of middle-aged female rats in the regulation of LH. Experimental Gerontology, 15, 201-207 (1980).

Kinsley, C., Meile, J., Wagner, C. K., Ghiraldi, L., Broida, J., and Svare, B. Prior intrauterine position influences body weight in male and female mice. Hormones and Behavior, 20, 201-211 (1986).

Kohama, S. G., Anderson, C. P., Osterburg, H. H., May, P. C., and Finch, C. E. Oral administration of estradiol to young C57BL/6J mice induces age-like neuroendocrine dysfunctions in the regulation of estrous cycles. Biology of Reproduction, 41, 227-232 (1989).

McDermott, N. J., Gandelman, R., and Reinisch, J. M. Contiguity to male fetuses influences ano-genital distance and time of vaginal opening in mice. Physiology and Behavior, 20, 661-663 (1978).

Meisel, R. L. and Ward, I. L. Fetal female rats are masculinized by male littermates located caudally in the uterus. Science, 213, 239-242 (1981).

Motelica-Heino, I., Castanier, M., Corbier, P., Edwards, D. A., and Roffi, J. Testosterone levels in plasma and testes of neonatal mice. Journal of Steroid Biochemistry, 3, 283-286 (1988).

Paup, D. C., Coniglio, L. P., and Clemens, L. G. Hormonal determinants in the development of masculine and feminine behavior in the female hamster. Behavioral Biology, 10, 353-363 (1974).

Quadagno, D. M., McQuitty, C., McKee, J., Kowlliker, L., Wolfe, G., and Johnson, D. C. The effects of intrauterine position on competition and behavior in the mouse. Physiology and Behavior, 41, 639-642 (1987).

Richmond, G. and Sachs, B. D. Further evidence for masculinization of female rats by males located caudally in utero. Hormones and Behavior, 18, 484-490 (1984).

Rines, J. P. and vom Saal, F. S. Fetal effects on sexual behavior and aggression in young and old female mice treated with estrogen and testosterone. Hormones and Behavior, 18, 117-129 (1984).

Slob, A. K. and van der Schoot, P. Testosterone induced mounting behavior in adult female rats born in litters of different female to male ratios. Physiology and Behavior, 28, 1007-1010 (1982).

Stiff, M. E., Bronson, F. H., and Stetson, M. H. Plasma gonadotropins in prenatal and prepubertal female mice: disorganization of pubertal cycles in the absence of a male. Endocrinology, 94, 492-496 (1974).

Vandenbergh, J. G. Effect of the presence of a male on the sexual maturation of female mice. Endocrinology, 81, 345-349 (1967).

Vandenbergh, J. G. Hormones, pheromones, and behavior. In Hormonal Correlates of Behavior, vol. 2 (Eds. B. Eleftheriou and R. Sprott) Plenum Press, New York, 1974.

Vomachka, A. J. and Lisk, R. D. Androgen and estradiol levels in plasma and amniotic fluid of late gestational mouse and female hamsters: uterine position effects. Hormones and Behavior, 20, 181-193 (1986).

vom Saal, F. S. and Bronson, F. H. In utero proximity of female mouse fetuses to males: effect of reproductive performance during later life. Biology of Reproduction, 19, 842-853 (1978).

vom Saal, F. S. and Bronson, F. H. Variation in length of the estrous cycle in mice due to former intrauterine proximity to male fetuses. Biology of Reproduction, 22, 777-780 (1980).

vom Saal, F. S. Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. Journal of Reproduction and Fertility, 62, 633-650 (1981).

vom Saal, F. S., Pryor, S., and Bronson, F. H. Effects of prior intrauterine position and housing on oestrous cycle length in adolescent mice. Journal of Reproduction and Fertility, 62, 33-37 (1981).

vom Saal, F. S. The interaction of circulating oestrogens and androgens in regulating mammalian sexual differentiation. In Hormones and Behavior in Higher Vertebrates (Eds. J. Balthazart, E. Prove, and R. Gilles) Springer-Verlag, Berlin, 1983.

vom Saal, F. S. and Moyer, C. L. Prenatal effects of reproductive capacity during aging in female mice. Biology of Reproduction, 32, 1116-1126 (1985).

Whitsett, J. M., Bronson, F. H., Peters, P. J., and Hamilton, T. H. Neonatal organization of aggression in mice: correlation of critical period with uptake of hormone. Hormones and Behavior, 3, 11-21 (1972).

# Appendices

## PLATES

Plate 1: Estrus Phase - vaginal smear containing cornified, non-nucleated epithelium cells.

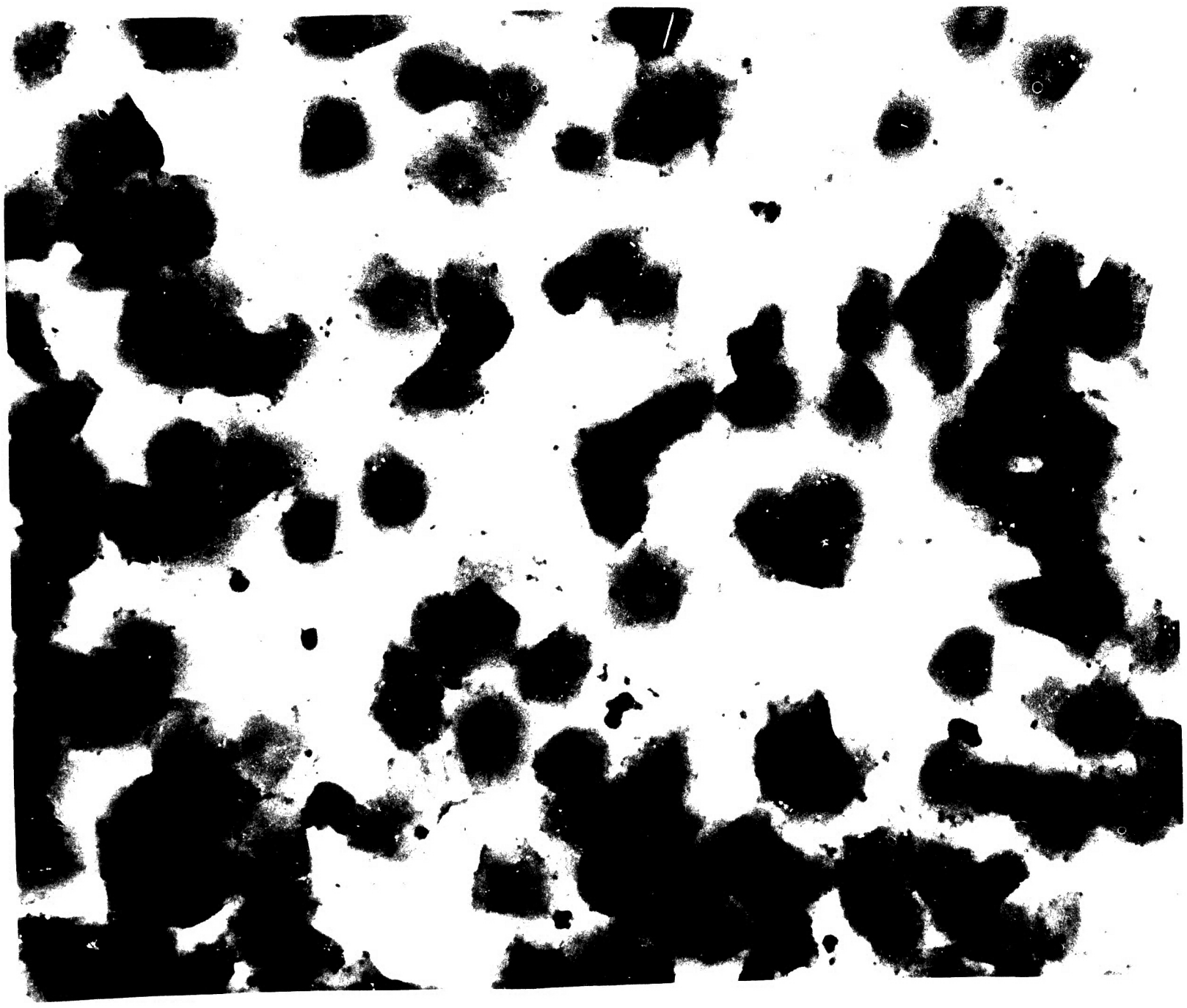


Plate 2: Metestrus Phase - vaginal smear containing cornified, non-nucleated epithelium cells infiltrated with leukocytes.

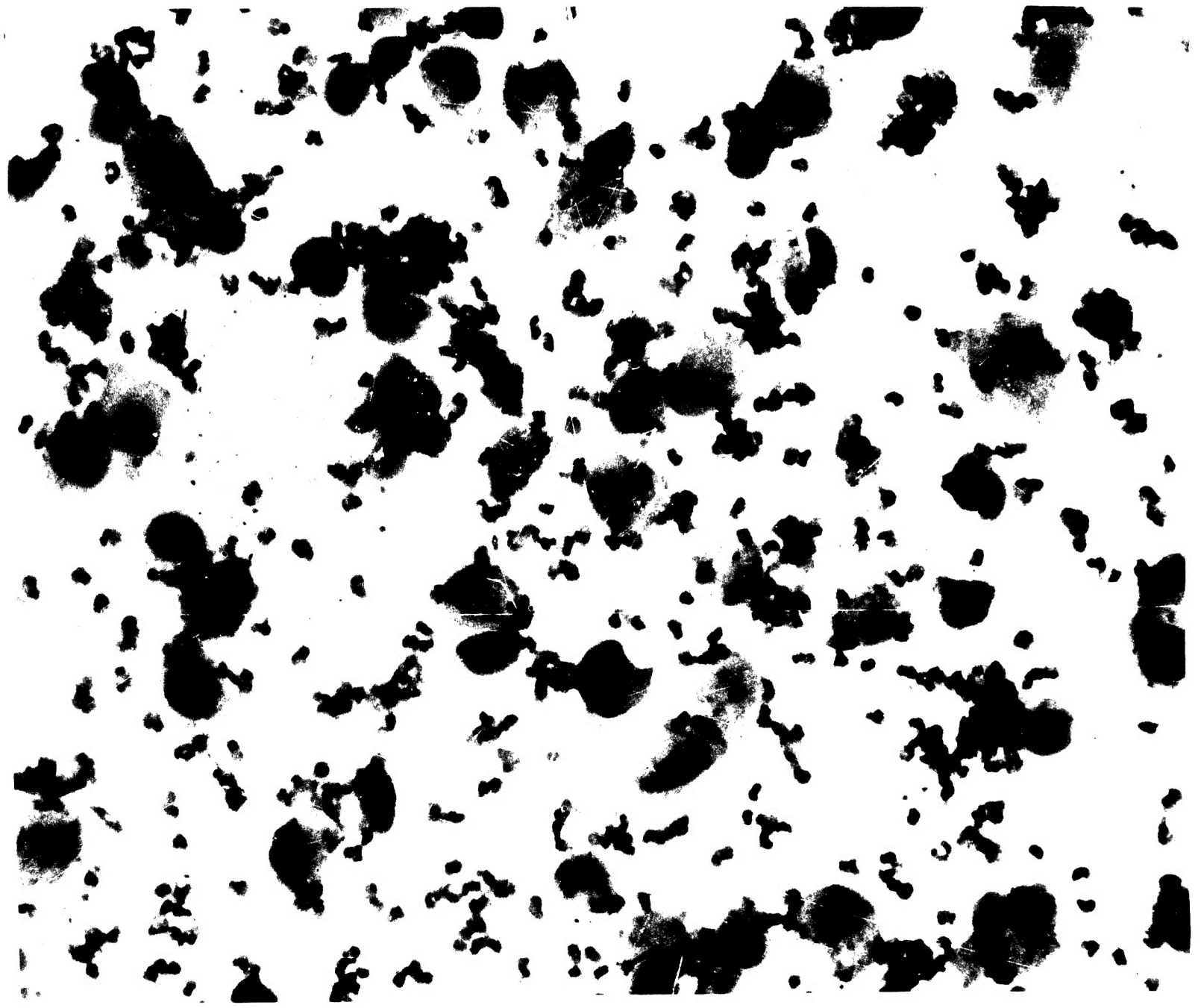


Plate 3: Diestrous Phase - vaginal smear containing leukocytes.

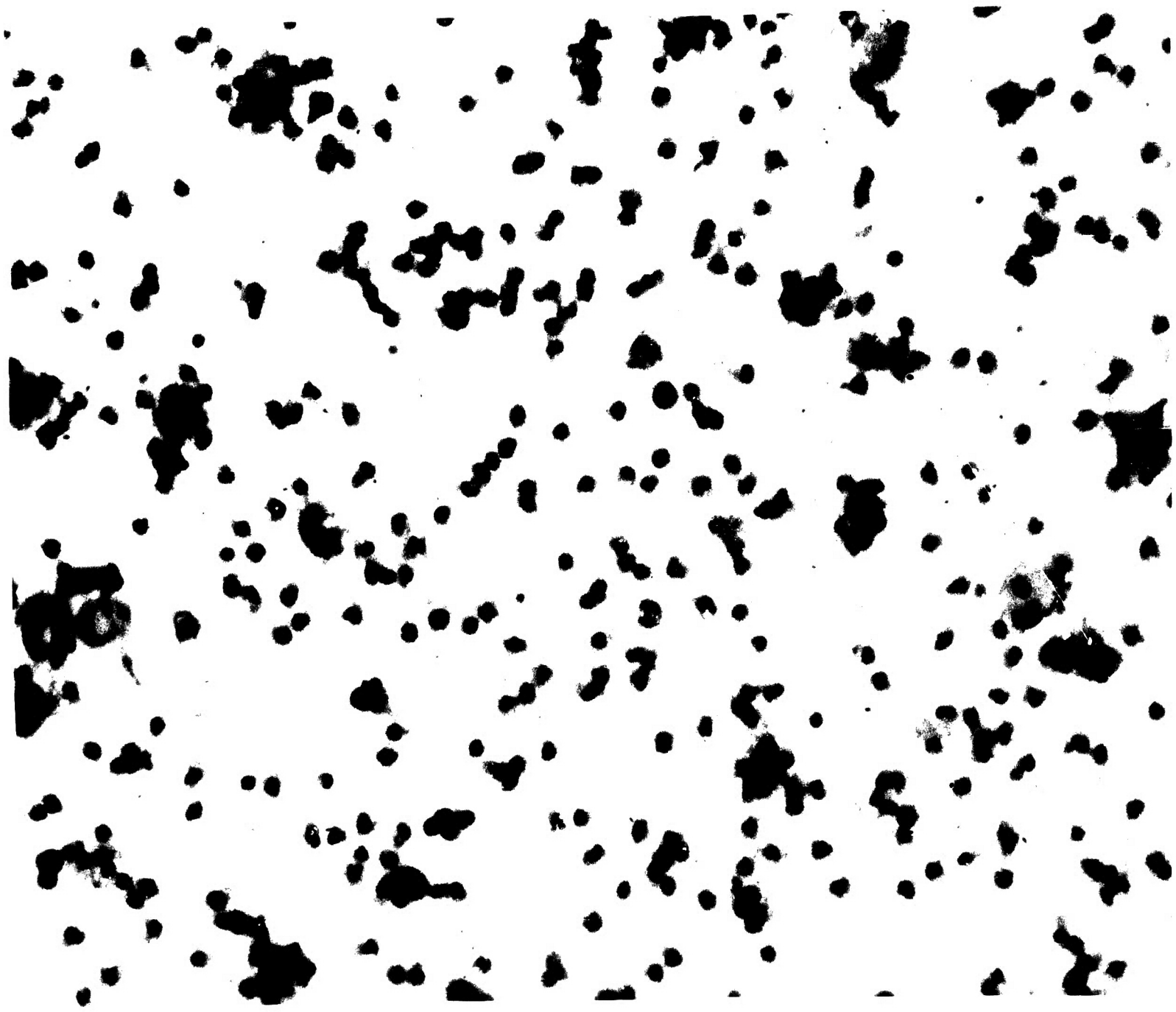


Plate 4: Proestrus Phase - vaginal smear containing nucleated epithelium cells.

# V I T A

Athena E. Cologer-Clifford

Born October 19, 1964


Current residence

739D East 7th Street
Bethlehem, PA 18015
(215) 691 1926

Work address

Psychology Department
Chandler-Ullmann #17
Lehigh University
Bethlehem, PA 18105
(215) 758 3630


Education

Lehigh University
Bethlehem, PA 18015
Fall 1988 - present

Towson State University
Towson, MD 21204
Spring 1984 - Spring 1987

Anne Arundel Comm. College
Arnold, MD 21012
Spring 1982 - Fall 1983

Employment

Graduate Orientation Coordinator
Graduate School
Lehigh University
Bethlehem, PA 18015
Summer 1991

Teaching Assistant
Psychology Department
Lehigh University
Fall 1988 - present

Clinical Research Assistant
Behavioral Pharmacology Research Unit
Johns Hopkins School of Medicine
Baltimore, MD 21224
1/87 - 8/88

## Fellowships

Graduate School Fellowship
Graduate School
Lehigh University
academic years 1989 - 1992

## Membership in Societies

American Psychological Society
Society for Neuroscience

## Research

Simon, N. G. and Cologer-Clifford, A. Uterine position does not influence morphology, behavioral sensitivity to testosterone, or hypothalamic androgen binding in CF-1 female mice. (Submitted for publication: Hormones and Behavior, 1991).

Cologer-Clifford, A. and Simon, N. G. Effects of uterine position on aggression in adult CK and CF-1 female mice. Eastern Psychological Association, 61, 9 (1990).